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(54) Title: DETECTION OF *CYP3A4* AND *CYP2C9* POLYMORPHISMS

(57) Abstract: The invention provides oligonucleotide primer pairs, sequence determination oligonucleotides, and kits for amplification and detection of novel single nucleotide polymorphisms in the 5' flanking regions of the *CYP3A4* and *CYP2C9* genes.



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DETECTION OF *CYP3A4* AND *CYP2C9* POLYMORPHISMS

The present invention is directed to methods of preparing biological samples for nucleic acid analysis using oligonucleotide primers suitable for amplification of the genes encoding the drug-metabolizing cytochrome P450 enzymes *CYP3A4* and *CYP2C19*.

BACKGROUND OF THE INVENTION

Xenobiotics are pharmacologically, endocrinologically, or toxicologically active substances foreign to a biological system. Most xenobiotics, including pharmaceutical agents, are metabolized through two successive reactions. Phase I reactions (functionalization reactions), include oxidation, reduction, and hydrolysis, in which a derivatizable group is added to the original molecule. Functionalization prepares the drug for further metabolism in phase II reactions. During phase II reactions (conjugative reactions, which include glucoronidation, sulfation, methylation and acetylation), the functionalized drug is conjugated with a hydrophilic group. The resulting hydrophilic compounds are inactive and excreted in bile or urine. Thus, metabolism can result in detoxification and excretion of the active substance. Alternatively, an inert xenobiotic may be metabolized to an active compound. For example, a pro-drug may be converted to a biologically active therapeutic or toxin.

The cytochrome P450 (CYP) enzymes are involved in the metabolism of many different xenobiotics. CYPs are a superfamily of heme-containing enzymes, found in eukaryotes (both plants and animals) and prokaryotes, and are responsible for Phase I reactions in the metabolic process. In total, over 500 genes belonging to the CYP superfamily have been described and divided into subfamilies, CYP1-CYP27. In humans, more than 35 genes and 7 pseudogenes have been identified. Members of three CYP gene families, CYP1, CYP2, and CYP3, are responsible for the majority of drug metabolism. The human CYPs which are of greatest clinical relevance for the metabolism of drugs and other xenobiotics are CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. The liver is the major site of activity of these enzymes, however CYPs are also expressed in other tissues.

The most important drug-metabolizing CYP enzyme is CYP3A4, which is the major CYP expressed in liver. Expression of the gene encoding CYP3A4 (*CYP3A4*) is inducible by many commonly used drugs, such as dexamethasone, rifampicin, and clotrimazole. CYP3A4 is estimated to metabolize more than 60% of all drugs in clinical use, including calcium channel blockers such as nifedipine, immunosuppressants such as cyclosporin A, macrolide antibiotics such as erythromycin, and steroid hormones. In addition, CYP3A4 metabolizes some carcinogens, and may be implicated in an individual's susceptibility to such toxins.

The existence of more than one form of the CYP3A4 enzyme is caused by polymorphisms in the gene which encodes the CYP3A4 enzyme (the gene being denoted in italics, as *CYP3A4*). In fact, almost 20 polymorphisms in the *CYP3A4* gene have been described (see <http://www.imm.ki.se/cypalleles/> for listing). The distribution of particular *CYP3A4* polymorphisms differs among ethnic groups, however, concomitant differences in CYP3A4 activity and responses to drugs which are CYP3A4 substrates remain to be investigated. *CYP3A4*1A* is the wild type gene, corresponding to the cDNA having GenBank Accession No. A18907 and the genomic DNA having GenBank Accession No. AF280107. A number of mutations in the 5' untranslated region of *CYP3A4* have been described. *CYP3A4*1B* is an A to G substitution at position -392. *CYP3A4*1C* is a T to G substitution at position -444. *CYP3A4*1D* is a C to A substitution at position -62. *CYP3A4*1E* is a T to A substitution at position -369. *CYP3A4*1F* is a C to G substitution at -747. The 5' flanking region of *CYP3A4* is set forth in SEQ ID NO:1 and in Figure 1.

WO 01/20025 discloses single nucleotide polymorphisms in various exons, introns, and in the 3' UTR of *CYP3A4*, as well as oligonucleotides for use in diagnosing and treating abnormal expression and/or function of this gene. WO 00/24926 discloses oligonucleotides for use in detecting an A to G point mutation at position -290 of *CYP3A4*. WO 99/13106 discloses polymorphisms in *CYP3A4*, including an A to G substitution at position -392 of the promoter, at the 7th position of the 10 bp NFSE, within oligonucleotides having sequences ACAAGGGCAAGAGAGAGGC (SEQ ID NO:2)

and ACAAGGGCAGGAGAGAGGC (SEQ ID NO:3), with polymorphic variants indicated in bold type.

U.S.Pat.No. 6,174,684 and corresponding WO 00/09752 disclose an A to G variant in the nifedipine-specific regulatory element located at positions -287 to -296 of *CYP3A4*, which is associated with increased risk of prostate cancer and with increased risk of developing leukemia after administration of an epipodophyllotoxin. U.S.Pat.No. 6,174,684 also discloses the oligonucleotides AGGGCAAGAG (SEQ ID NO:4) and AGGGCAGGAG (SEQ ID NO:5), with polymorphic variants indicated in bold type. Rebbeck, *et al.* (1998) *J. Natl. Cancer Inst.* **90**, 1225-1229 also describes this association between prostate cancer, leukemia, and the A to G mutation.

Kuehl, *et al.* (2001) *Nature Genetics* 27, 383-391 discloses mutations at positions -341, -288, and -43 of the *CYP3A4* promoter, none of which were associated with altered *CYP3A4* activity. Kuehl, *et al.* also discloses differential distribution of these polymorphisms among Caucasians and African Americans.

A second important CYP enzyme is *CYP2C9*, which is active in hydroxylation of such drugs as tolbutamide, phenytoin, S-warfarin, diclofenac, ibuprofen, and losarten. The sequence of *CYP2C9* is set forth in SEQ ID NO:6. Six variants in *CYP2C9* are described on the CYP web site, and another six variant designations are listed without descriptions. The *CYP2C9*1* variant is designated as the wild type. Four of the five polymorphic *CYP2C9* forms described contain mutations in the coding regions of the gene that result in decreased *in vitro* activity, and the remaining variant, *CYP2C9*6*, is a deletion of an A at position 818 which results in a frame shift.

WO00/12757 discloses primer extension assays and kits for detection of the single nucleotide polymorphisms *CYP2C9*2* and *CYP2C9*3*, both of which result in amino acid substitutions.

On the basis of ability of metabolize a marker drug such as nifedipine for *CYP3A4* or S-warfarin for *CYP2C9*, individuals may be characterized as poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) or ultra extensive metabolizers (UEM or UM) for *CYP3A4* or *CYP2C9* substrates, respectively. Poor metabolizers retain the substrate in their bodies for a relatively long period of time,

and are susceptible to toxicity and side effects at “normal” dosages. Ultraextensive metabolizers clear the substrate from their bodies quickly, and require higher than “normal” dosages to achieve a therapeutic effect. Intermediate and extensive metabolizers retain the substrate in their bodies for times between those of PMs and UEMs, and are more likely to respond to “normal” dosages of the drug. However, individuals characterized as IM or EM may differ in drug clearance by as much as 10-fold, and variations in toxicity, side effects, and efficacy for a particular drug may occur among these individuals. However, administration of such drugs to determine an individual’s metabolic capacity may in itself be dangerous, exposing the individual to potential toxic side effects.

A need remains for methods of preparing biological samples that contain the 5' flanking regions of *CYP3A4* or *CYP2C9*, so that this information may be used to predict differential capacities for metabolizing *CYP3A4* and *CYP2C9* substrates among individuals.

SUMMARY OF THE INVENTION

The present inventors have discovered a novel single nucleotide polymorphism in the 5' flanking region of *CYP3A4*, and six novel polymorphisms in the 5' flanking region of *CYP2C9*. Oligonucleotides have been devised for amplification of the polymorphic regions corresponding to these polymorphisms. These oligonucleotides may be used to prepare biological samples for further analysis of the 5' flanking regions of these genes. The inventors have also devised sequence determination oligonucleotides for use as probes for the novel single nucleotide polymorphisms in *CYP3A4* and *CYP2C9*.

In one embodiment, the invention provides an oligonucleotide primer pair suitable for amplifying a polymorphic region of a 5' flanking region of a *CYP3A4* gene, wherein the polymorphic region corresponds to position 461 of SEQ ID NO:1, which position may also be described as position -644 from the transcription start site of the *CYP3A4* gene.

In another embodiment, the invention provides a sequence determination oligonucleotide for detecting a polymorphic site in a 5' flanking region of a *CYP3A4* gene, said oligonucleotide being complementary to the polymorphic region corresponding to position 461 of SEQ ID NO:1.

In another embodiment, the invention provides a kit for amplification and/or detection of a polymorphic region of the 5' flanking region of a *CYP3A4* gene, said kit comprising at least one oligonucleotide primer pair capable of amplifying the region corresponding to position 461 of SEQ ID NO:1.

In another embodiment, the invention provides an oligonucleotide primer pair suitable for amplifying a polymorphic region of a 5' flanking region of a *CYP2C9* gene, wherein the polymorphic region corresponds to position 957 of SEQ ID NO:6; position 1049 of SEQ ID NO:6; position 1164 of SEQ ID NO:6; position 1526 of SEQ ID NO:6; position 1661 of SEQ ID NO:6; and position 1662 of SEQ ID NO:6. Position 957 of SEQ ID NO:6 may also be described as position -1189 from the transcription start site of the *CYP3C9* gene; position 1049 of SEQ ID NO:6 may also be described as position -1097 from the transcription start site; position 1164 of SEQ ID NO:6 may also be described as position -982 from the transcription start site; position 1526 of SEQ ID

NO:6 may also be described as position -620 from the transcription start site; position 1661 of SEQ ID NO:6 may also be described as position -485 from the transcription start site; and position 1662 of SEQ ID NO:6 may also be described as position -484 from the transcription start site.

5 In yet another embodiment, the invention provides a sequence determination oligonucleotide for detecting a polymorphic site in a 5' flanking region of a *CYP2C9* gene, said oligonucleotide comprising a sequence selected from the group consisting of an oligonucleotide complementary to the polymorphic region corresponding to position 957 of SEQ ID NO:6; an oligonucleotide complementary to the polymorphic region
10 corresponding to position 1049 of SEQ ID NO:6; an oligonucleotide complementary to the polymorphic region corresponding to position 1164 of SEQ ID NO:6; an oligonucleotide complementary to the polymorphic region corresponding to position 1526 of SEQ ID NO:6; an oligonucleotide complementary to the polymorphic region corresponding to position 1661 of SEQ ID NO:6; and an oligonucleotide complementary
15 to the polymorphic region corresponding to position 1662 of SEQ ID NO:6.

 In another embodiment, the invention provides a kit for amplification and/or detection of a polymorphic region corresponding to at least one polymorphic region in the 5' flanking region of the *CYP2C9* gene, said region being selected from the group consisting of position 957 of SEQ ID NO:6; position 1049 of SEQ ID NO:6; position
20 1164 of SEQ ID NO:6; position 1526 of SEQ ID NO:6; position 1661 of SEQ ID NO:6; and position 1662 of SEQ ID NO:6.

BRIEF DESCRIPTION OF THE DRAWINGS

25 Figure 1 shows the sequence of the 5' flanking region of the *CYP3A4* gene as set forth in SEQ ID NO:1, with the novel polymorphic site underlined and highlighted in bold.

 Figure 2 shows the sequence of the 5' flanking region of the *CYP2C9* gene as set forth in SEQ ID NO:6, with the novel polymorphic sites underlined and highlighted in
30 bold.

DETAILED DESCRIPTION OF THE INVENTION

The U.S. patents and publications referenced herein are hereby incorporated by reference.

For the purposes of the invention, certain terms are defined as follows.

5 “Gene” is defined as the genomic sequence of the *CYP2C19* gene.

 “Oligonucleotide” means a nucleic acid molecule preferably comprising from about 8 to about 50 covalently linked nucleotides. More preferably, an oligonucleotide of the invention comprises from about 8 to about 35 nucleotides. Most preferably, an oligonucleotide of the invention comprises from about 10 to about 25 nucleotides. In
10 accordance with the invention, the nucleotides within an oligonucleotide may be analogs or derivatives of naturally occurring nucleotides, so long as oligonucleotides containing such analogs or derivatives retain the ability to hybridize specifically within the polymorphic region containing the targeted polymorphism. Analogs and derivatives of naturally occurring oligonucleotides within the scope of the present invention are
15 exemplified in U.S. Pat. Nos. 4,469,863; 5,536,821; 5,541,306; 5,637,683; 5,637,684; 5,700,922; 5,717,083; 5,719,262; 5,739,308; 5,773,601; 5,886,165; 5,929,226; 5,977,296; 6,140,482; WO 00/56746; WO 01/14398, and the like. Methods for synthesizing oligonucleotides comprising such analogs or derivatives are disclosed, for example, in the patent publications cited above and in U.S. Pat. Nos. 5,614,622; 5,739,314; 5,955,599;
20 5,962,674; 6,117,992; in WO 00/75372, and the like. The term “oligonucleotides” as defined herein also includes compounds which comprise the specific oligonucleotides disclosed herein, covalently linked to a second moiety. The second moiety may be an additional nucleotide sequence, for example, a tail sequence such as a polyadenosine tail or an adaptor sequence, for example, the phage M13 universal tail sequence, and the like.
25 Alternatively, the second moiety may be a non-nucleotidic moiety, for example, a moiety which facilitates linkage to a solid support or a label to facilitate detection of the oligonucleotide. Such labels include, without limitation, a radioactive label, a fluorescent label, a chemiluminescent label, a paramagnetic label, and the like. The second moiety may be attached to any position of the specific oligonucleotide, so long as the

oligonucleotide retains its ability to hybridize to the polymorphic regions described herein.

A polymorphic region as defined herein is a portion of a genetic locus that is characterized by at least one polymorphic site. A genetic locus is a location on a chromosome which is associated with a gene, a physical feature, or a phenotypic trait. A polymorphic site is a position within a genetic locus at which at least two alternative sequences have been observed in a population. A polymorphic region as defined herein is said to "correspond to" a polymorphic site, that is, the region may be adjacent to the polymorphic site on the 5' side of the site or on the 3' side of the site, or alternatively may contain the polymorphic site. A polymorphic region includes both the sense and antisense strands of the nucleic acid comprising the polymorphic site, and may have a length of from about 100 to about 5000 base pairs. For example, a polymorphic region may be all or a portion of a regulatory region such as a promoter, 5' UTR, 3' UTR, an intron, an exon, or the like. A polymorphic or allelic variant is a genomic DNA, cDNA, mRNA or polypeptide having a nucleotide or amino acid sequence that comprises a polymorphism. A polymorphism is a sequence variation observed at a polymorphic site, including nucleotide substitutions (single nucleotide polymorphisms or SNPs), insertions, deletions, and microsatellites. Polymorphisms may or may not result in detectable differences in gene expression, protein structure, or protein function. Preferably, a polymorphic region of the present invention has a length of about 1000 base pairs. More preferably, a polymorphic region of the invention has a length of about 500 base pairs. Most preferably, a polymorphic region of the invention has a length of about 200 base pairs.

A haplotype as defined herein is a representation of the combination of polymorphic variants in a defined region within a genetic locus on one of the chromosomes in a chromosome pair. A genotype as used herein is a representation of the polymorphic variants present at a polymorphic site.

The PCR primer pairs of the invention are capable of amplifying the polymorphic region corresponding to position 461 of SEQ ID NO:1, or any of the polymorphic regions corresponding to position 957 of SEQ ID NO:6; position 1049 of SEQ ID NO:6; position

1164 of SEQ ID NO:6; position 1526 of SEQ ID NO:6; position 1661 of SEQ ID NO:6; and position 1662 of SEQ ID NO:6. Specific oligonucleotide primer pairs of the invention, for amplifying position 461 of SEQ ID NO:1, may comprise sequences selected from the group consisting of SEQ ID NO:7 and SEQ ID NO:8; and SEQ ID NO:9 and SEQ ID NO:10. For amplifying only position 957 of SEQ ID NO:6, an oligonucleotide primer pair comprising the sequences set forth in SEQ ID NO:19 and SEQ ID NO:20 may be used. Alternatively, positions 957 and 1049 of SEQ ID NO:6 may be amplified using an oligonucleotide primer pair comprising the sequences set forth in SEQ ID NO:21 and SEQ ID NO:22; or positions 957, 1049, and 1164 may be amplified using an oligonucleotide primer pair comprising the sequences set forth in SEQ ID NO:23 and SEQ ID NO:24. Position 1164 of SEQ ID NO:6 may also be amplified using an oligonucleotide primer pair comprising the sequences set forth in SEQ ID NO:25 and SEQ ID NO:26. Positions 1526, 1661, and 1662 of SEQ ID NO:6 may be amplified using an oligonucleotide primer pair comprising the sequences set forth in SEQ ID NO:27 and SEQ ID NO:28. Positions 1661 and 1662 of SEQ ID NO:6 may be amplified using an oligonucleotide primer pair selected from the group consisting of an oligonucleotide primer pair comprising the sequences set forth in SEQ ID NO:29 and SEQ ID NO:30 and an oligonucleotide primer pair comprising the sequences set forth in SEQ ID NO:31 and SEQ ID NO:32.

Each of the PCR primer pairs of the invention may be used in any PCR method. For example, a PCR primer pair of the invention may be used in the methods disclosed in U.S.Pat.Nos. 4,683,195; 4,683,202, 4,965,188; 5,656,493; 5,998,143; 6,140,054; WO 01/27327; WO 01/27329; and the like. The PCR pairs of the invention may also be used in any of the commercially available machines that perform PCR, such as any of the GeneAmp® Systems available from Applied Biosystems.

The oligonucleotides of the invention may be used to determine the sequence of the polymorphic regions of SEQ ID NO:1 or SEQ ID NO:6 as defined herein. In one embodiment, an oligonucleotide of the invention comprises a sequence selected from the group consisting of SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID NO:17; and SEQ ID NO:18, for determining

the sequence of the novel polymorphic region of *CYP3A4* corresponding to position 461 of SEQ ID NO:1. In another embodiment, for determining the sequence of the polymorphic region of *CYP2C9* corresponding to position 957 of SEQ ID NO:6, an oligonucleotide of the invention comprises a sequence selected from the group consisting of SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:43; SEQ ID NO:44; SEQ ID NO:53; SEQ ID NO:58; SEQ ID NO:63; and SEQ ID NO:68. In another embodiment, for determining the sequence of the polymorphic region of *CYP2C9* corresponding to position 1049 of SEQ ID NO:6, an oligonucleotide of the invention comprises a sequence selected from the group consisting of SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:45; SEQ ID NO:46; SEQ ID NO:54; SEQ ID NO:59; SEQ ID NO:64; and SEQ ID NO:69. In another embodiment, for determining the sequence of the polymorphic region of *CYP2C9* corresponding to position 1164 of SEQ ID NO:6, an oligonucleotide of the invention comprises a sequence selected from the group consisting of SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:45; SEQ ID NO:48; SEQ ID NO:55; SEQ ID NO:60; SEQ ID NO:65; and SEQ ID NO:70. In another embodiment, for determining the sequence of the polymorphic region of *CYP2C9* corresponding to position 1526 of SEQ ID NO:6, an oligonucleotide of the invention comprises a sequence selected from the group consisting of SEQ ID NO:39; SEQ ID NO:40; SEQ ID NO:49; SEQ ID NO:50; SEQ ID NO:56; SEQ ID NO:61; SEQ ID NO:66; and SEQ ID NO:71. In another embodiment, for determining the sequences of the polymorphic region of *CYP2C9* corresponding to either of positions 1661 or 1662 of SEQ ID NO:6, an oligonucleotide of the invention comprises a sequence selected from the group consisting of SEQ ID NO:41; SEQ ID NO:42; SEQ ID NO:51; SEQ ID NO:52; SEQ ID NO:57; SEQ ID NO:62; SEQ ID NO:67; and SEQ ID NO:72.

Those of ordinary skill will recognize that oligonucleotides complementary to the polymorphic regions described herein must be capable of hybridizing to the polymorphic regions under conditions of stringency such as those employed in primer extension-based sequence determination methods, restriction site analysis, nucleic acid amplification methods, ligase-based sequencing methods, methods based on enzymatic detection of mismatches, microarray-based sequence determination methods, and the like. The

oligonucleotides of the invention may be synthesized using known methods and machines, such as the ABI™3900 High Throughput DNA Synthesizer and the Expedite™ 8909 Nucleic Acid Synthesizer, both of which are available from Applied Biosystems (Foster City, CA).

5 The oligonucleotides of the invention may be used, without limitation, as *in situ* hybridization probes or as components of diagnostic assays. Numerous oligonucleotide-based diagnostic assays are known. For example, primer extension-based nucleic acid sequence detection methods are disclosed in U.S.Pat.Nos. 4,656,127; 4,851,331; 5,679,524; 5,834,189; 5,876,934; 5,908,755; 5,912,118; 5,976,802; 5,981,186; 6,004,744; 10 6,013,431; 6,017,702; 6,046,005; 6,087,095; 6,210,891; WO 01/20039; and the like. Primer extension-based nucleic acid sequence detection methods using mass spectrometry are described in U.S.Pat.Nos. 5,547,835; 5,605,798; 5,691,141; 5,849,542; 5,869,242; 5,928,906; 6,043,031; 6,194,144, and the like. The oligonucleotides of the invention are also suitable for use in ligase-based sequence determination methods such as those disclosed in U.S.Pat.Nos. 5,679,524 and 5,952,174, WO 01/27326, and the like. 15 The oligonucleotides of the invention may be used as probes in sequence determination methods based on mismatches, such as the methods described in U.S.Pat.Nos. 5,851,770; 5,958,692; 6,110,684; 6,183,958; and the like. In addition, the oligonucleotides of the invention may be used in hybridization-based diagnostic assays such as those described 20 in U.S.Pat.Nos. 5,891,625; 6,013,499; and the like.

 The oligonucleotides of the invention may also be used as components of a diagnostic microarray. Methods of making and using oligonucleotide microarrays suitable for diagnostic use are disclosed in U.S.Pat.Nos. 5,492,806; 5,525,464; 5,589,330; 5,695,940; 5,849,483; 6,018,041; 6,045,996; 6,136,541; 6,142,681; 6,156,501; 6,197,506; 25 6,223,127; 6,225,625; 6,229,911; 6,239,273; WO 00/52625; WO 01/25485; WO 01/29259; and the like.

 The invention is also embodied in a kit comprising at least one oligonucleotide primer pair of the invention. When the kit is used for amplification and detection of *CYP3A4* polymorphisms, it will comprise an oligonucleotide primer pair suitable for 30 amplification of the polymorphic region corresponding to position 461 of SEQ ID NO:1.

Specific primer pairs in this embodiment are selected from the group consisting of SEQ ID NO:7 and SEQ ID NO:8; and SEQ ID NO:9 and SEQ ID NO:10. This embodiment of the kit of the invention may optionally comprise a sequence determination oligonucleotide selected from the group consisting of SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID NO:17; and SEQ ID NO:18.

When the kit of the invention is used for amplification and detection of polymorphisms in the 5' flanking region of *CYP2C9*, it will comprise at least one oligonucleotide primer pair, wherein the primer pair is capable of amplifying a polymorphic region selected from the group consisting of the polymorphic region corresponding to position 957 of SEQ ID NO:6; the polymorphic region corresponding to position 1049 of SEQ ID NO:6; the polymorphic region corresponding to position 1164 of SEQ ID NO:6; the polymorphic region corresponding to position 1526 of SEQ ID NO:6; the polymorphic region corresponding to position 1661 of SEQ ID NO:6; and the polymorphic region corresponding to position 1662 of SEQ ID NO:6. This embodiment may optionally further comprise a sequence determination oligonucleotide for detecting a polymorphic variant at any or all of the polymorphic sites corresponding to positions 957, 1049, 1164, 1526, 1661 and 1662 of SEQ ID NO:6.

The kit of the invention may also comprise a polymerizing agent, for example, a thermostable nucleic acid polymerase such as those disclosed in U.S.Pat.Nos. 4,889,818; 6,077,664, and the like. The kit of the invention may also comprise chain elongating nucleotides, such as dATP, dTTP, dGTP, dCTP, and dITP, including analogs of dATP, dTTP, dGTP, dCTP and dITP, so long as such analogs are substrates for a thermostable nucleic acid polymerase and can be incorporated into a growing nucleic acid chain. The kit of the invention may also include chain terminating nucleotides such as ddATP, ddTTP, ddGTP, ddCTP, and the like. In a preferred embodiment, the kit of the invention comprises at least two oligonucleotide primer pairs, a polymerizing agent, chain elongating nucleotides, at least two sequence determination oligonucleotides and at least one chain terminating nucleotide. The kit of the invention may optionally include buffers, vials, microtiter plates, and instructions for use.

The examples set forth below are provided as illustration and are not intended to limit the scope and spirit of the invention as specifically embodied therein.

EXAMPLE 1

IDENTIFICATION OF *CYP3A4* POLYMORPHISM

The study was performed in accordance with the principles stated in the Declaration of Helsinki as reviewed in Tokyo 1975 and Venice 1983, Hong Kong 1989 and Somerset West 1996. Ten samples (Swedish Caucasians) were selected and used for identification of polymorphisms in the 5' flanking region of *CYP3A4*.

White blood cells isolated from a blood sample drawn from the brachial vein serve as the source of the genomic DNA for the analyses. The DNA was extracted by guanidine thiocyanate method or QIAamp Blood Kit (QIAGEN, Venlo, The Netherlands). The genes included in the study were amplified by PCR and the DNA sequences were determined by full sequencing. All genetic analyses were performed according to Good Laboratory Practice and Standard Operating Procedures. Case Report Forms were designed and used for clinical and genetic data collection. Data was entered and stored in a relational database at Gemini Genomics AB, Uppsala. To secure consistency between the Case Report Forms and the database, data was checked either by double data entry or proofreading. After a Clean File was declared the database was protected against changes. By using the program Stat/TransferTM the database was transferred to SAS data sets. The SASTM system was used for tabulations and statistical evaluations. Genotypes were also correlated against the metabolic ratio.

PCR-fragments were amplified with TaqGOLD polymerase (Applied Biosystems) using Robocycler (Stratagene) or GeneAmp PCR system 9700 (Applied Biosystems). Preferentially, the amplified fragments were 300-400 bp, and the region to be read did not exceed 300 bp. PCR reactions were carried out according to the basic protocol set forth in Table 1, with modifications as indicated in Table 2 for specific primer pairs, which are shown in Table 3. For the GeneAmp PCR 9700 machine the profile used was 10 minutes at 95°, 40 x (45 seconds at 90°, 45 seconds at 60°, 45 seconds at 72°), 5 minutes at 72° and 22° until removed.

Table 1

| Solution | Stock Concentration | PCR (μl) |
|---------------------|----------------------------|-----------------|
| H ₂ O | . | 33.2 |
| PCR buffer | 10x | 5.0 |
| MgCl ₂ | 25 mM | 2.0 |
| dNTP | 2.5 mM | 2.5 |
| primer 1 | 10μM | 1.0 |
| primer 2 | 10μM | 1.0 |
| Taq-gold polymerase | 5 μ/μl | 0.3 |
| DNA samples | 2 ng/μl | 5.0 |
| TOTAL | | 50.0 |

Table 2

| SEQ ID NO:s | Polymorphic Site | Modification from basic protocol (Table 1) | Detection method |
|--------------------|-------------------------|---|-------------------------|
| 7, 8 | 461 | 62° annealing temperature | Full sequencing |
| 9, 10 | 461 | 3 μl MgCl ₂ , 58° annealing temperature, 50 cycles | Full sequencing |

5

Table 3

| Polymorphic Site | Primer Pair |
|-------------------------|--|
| 461 | SEQ ID NO:7 CCAGCCTGAAAGTGCAGAGA SEQ ID NO:8 TCTTAGAGTCTTTCCTCACCAAAC |
| 461 | SEQ ID NO:9 CATGCCCTGTCTCTCCTTTA SEQ ID NO:10 CCATCCCCTTCATGCAATC |

10 The optimized condition specified in Table 2 were required to distinguish *CYP3A4* from the closely related gene-family members *CYP3A5*, and *CYP3A7*. Use of the basic protocol will lead to problems when amplifying *CYP3A4*-specific amplicons of 300-400 bp

containing the polymorphisms of interest, unless a nested PCR approach is carried out. The nested PCR approach was not used because of the high risk of contamination when using a nested PCR approach and the high risk of typing errors as a consequence. The modifications shown in Table 2 were optimized and reaction parameters were balanced in such a way that nested PCR was avoided.

For full sequencing, one of the PCR-primers in a primer pair was designed for sequencing by addition of a 29 nucleotide tail complementary to M13 at its 5'-end, namely the nucleotides AGTCACGACGTTGTAAAACGACGGCCAGT. Thus, the entire PCR-product was sequenced from the tailed PCR-primer.

The additional oligonucleotides set forth in Tables 4 through 7 were identified as being suitable for detection of the SNP at positions 461 of the 5' flanking region of the *CYP3A4* gene as depicted in SEQ ID NO:1.

Table 4 sets forth oligonucleotides representing the coding (sense) strand complementary to the polymorphic region corresponding to the novel polymorphism found in the study population. The underlined letter indicates polymorphic position in the sequence context. All sequences are shown in 5' to 3' direction.

Table 4

| Polymorphic Site | Sequence | Note |
|------------------|--|------------------------|
| 461 | SEQ ID NO:11: AGCAC <u>C</u> CTGGT SEQ ID NO:12: AGCAC <u>G</u> CTGGT | C variant G variant |

Table 5 sets forth oligonucleotides representing the non-coding (anti-sense) strand complementary to the polymorphic region corresponding to the novel polymorphism found in the study population. The underlined letter indicates polymorphic position in the sequence context. All sequences are shown in 5' to 3' direction.

Table 5

| Polymorphic Site | Sequence | Note |
|------------------|--|--|
| 461 | SEQ ID NO:13: ACCAG <u>G</u> GTGCT SEQ ID NO:14: ACCAG <u>C</u> GTGCT | Antisense G variant Antisense C variant |

The sequences of Table 6 represent the 5'-sequence to the novel polymorphic site on the coding (sense) strand (SEQ ID NO: 15) and non-coding (anti-sense) strand (SEQ ID NO:s 16). All sequences are shown in 5' to 3' direction.

5

Table 6

| Polymorphic Site | Sequence | Note |
|------------------|--|--------------------------|
| 461 | SEQ ID NO:15: GTGTGTACAGC SEQ ID NO:16: GCTGTACACAC | Sense 5' Antisense 5' |

The sequences of Table 7 represent the 3'-sequence to the novel polymorphic site on the non-coding (anti-sense) strand (SEQ ID NO:17) and the coding (sense) strand (SEQ ID NO:18). All sequences are shown in 5' to 3' direction.

10

Table 9

| Polymorphic Site | Sequence | Note |
|------------------|--|--------------------------|
| 461 | SEQ ID NO:17: TGGTCCCTACC SEQ ID NO:18: GGTAGGGACCA | Antisense 3' Sense 3' |

15

EXAMPLE 2

IDENTIFICATION OF *CYP2C9* POLYMORPHISMS

The study was performed in accordance with the principles stated in the Declaration of Helsinki as reviewed in Tokyo 1975 and Venice 1983, Hong Kong 1989 and Somerset West 1996. Ten samples (Swedish Caucasians) were selected and used for identification of polymorphisms in the 5' flanking region of *CYP2C9*.

White blood cells isolated from a blood sample drawn from the brachial vein serve as the source of the genomic DNA for the analyses. The DNA is extracted by guanidine thiocyanate method or QIAamp Blood Kit (QIAGEN, Venlo, The Netherlands). The genes included in the study were amplified by PCR and the DNA sequences were determined by full sequencing. All genetic analyses were performed according to Good Laboratory Practice and Standard Operating Procedures. Case Report Forms were designed and used for clinical and genetic data collection. Data was entered and stored in a relational database at Gemini Genomics AB, Uppsala. To secure consistency between the Case Report Forms and the database, data was checked either by double data entry or proofreading. After a Clean File was declared the database was protected against changes. By using the program Stat/Transfer™ the database was transferred to SAS data sets. The SAS™ system was used for tabulations and statistical evaluations. Genotypes were also correlated against the metabolic ratio.

PCR-fragments were amplified with TaqGOLD polymerase (Applied Biosystems) using Robocycler (Stratagene) or GeneAmp PCR system 9700 (Applied Biosystems). Preferentially, the amplified fragments were 300-400 bp, and the region to be read did not exceed 300 bp. PCR reactions were carried out according to the basic protocol set forth in Table 10, with modifications as indicated in Table 11 for specific primer pairs, which are shown in Table 12. For the GeneAmp PCR 9700 machine the profile used was 10 minutes at 95°, 40 x (45 seconds at 90°, 45 seconds at 60°, 45 seconds at 72°), 5 minutes at 72° and 22° until removed.

Table 10

| Solution | Stock Concentration | PCR (μl) |
|---------------------|----------------------------|-----------------|
| H ₂ O | | 33.2 |
| PCR buffer | 10x | 5.0 |
| MgCl ₂ | 25 mM | 2.0 |
| dNTP | 2.5 mM | 2.5 |
| primer 1 | 10μM | 1.0 |
| primer 2 | 10μM | 1.0 |
| Taq-gold polymerase | 5 μ/μl | 0.3 |
| DNA samples | 2 ng/μl | 5.0 |
| TOTAL | | 50.0 |

Table 11

| SEQ ID NO:s | Polymorphic Site | Modification from basic protocol (Table 10) | Detection method |
|--------------------|-------------------------|--|-------------------------|
| 19, 20 | 957 | 58° annealing temperature | Full sequencing |
| 21, 22 | 957 & 1049 | 3 μl MgCl ₂ , 62° annealing temperature | Full sequencing |
| 23, 24 | 957, 1049 & 1164 | 58° annealing temperature | Full sequencing |
| 25, 26 | 1164 | 3 μl MgCl ₂ , 62° annealing temperature , 50 cycles | Full sequencing |
| 27, 28 | 1526, 1661 & 1662 | | Full sequencing |
| 29, 30 | 1661 & 1662 | 3 μl MgCl ₂ , 62° annealing temperature , 50 cycles | Full sequencing |
| 31, 32 | 1661 & 1662 | | Full sequencing |

5

10

Table 12

| Polymorphic Site | Primer Pair | |
|-------------------|--------------|-----------------------------|
| 957 | SEQ ID NO:19 | CACTAGGGAATTTAGAACAAATATG |
| | SEQ ID NO:20 | GCACAGAAAGCAAAGGAAATTAT |
| 957 & 1049 | SEQ ID NO:21 | TGTATTTAGATCCTCAACTCAGTATGT |
| | SEQ ID NO:22 | GGATCTCCCTTCTCCATCACT |
| 957, 1049 & 1164 | SEQ ID NO:23 | GGTCCATTTAGTGATTTCCCTAC |
| | SEQ ID NO:24 | ATACACCACATTTATTCTGTTCATA |
| 1164 | SEQ ID NO:25 | CCAAATTTTTCCCTCAGTTACA |
| | SEQ ID NO:26 | TTGGTGCCACACAGCTCATA |
| 1526, 1661 & 1662 | SEQ ID NO:27 | GCCTTCAGGAATTTTTTTTA |
| | SEQ ID NO:28 | CCAGTTGGGAATATATGATTTAACA |
| 1661 & 1662 | SEQ ID NO:29 | GCTGCTGTATTTTTAGTAGGCTATA |
| | SEQ ID NO:30 | CGTTCCATTGTCCACTCTGTAC |
| 1661 & 1662 | SEQ ID NO:31 | TCAAGGCAGCTCTGGTGTA |
| | SEQ ID NO:32 | AGTTGGGAATATATGATTTAACAGA |

The optimized condition specified in Table 11 were required to distinguish *CYP2C9* from the closely related gene-family members *CYP2C8*, *CYP2C18* and *CYP2C19*. Use of the basic protocol will lead to problems when amplifying *CYP2C9*-specific amplicons of 300-400 bp containing the polymorphisms of interest, unless a nested PCR approach is carried out. The nested PCR approach was not used because of the high risk of contamination when using a nested PCR approach and the high risk of typing errors as a consequence. The modifications shown in Table 11 were optimized and reaction parameters were balanced in such a way that nested PCR was avoided.

For full sequencing, one of the PCR-primers in a primer pair was designed for sequencing by addition of a 29 nucleotide tail complementary to M13 at its 5'-end, namely the nucleotides AGTCACGACGTTGTAAAACGACGGCCAGT. Thus, the entire PCR-product was sequenced from the tailed PCR-primer.

The additional oligonucleotides set forth in Tables 13 through 16 were identified as being suitable for detection of the SNPs at positions 957, 1049, 1164, 1526, 1661 and/or 1662 of the 5' flanking region of the *CYP2C9* gene as depicted in SEQ ID NO:6.

Table 13 sets forth oligonucleotides representing the coding (sense) strand complementary to the polymorphic region corresponding to the polymorphisms found in the study population. The underlined letter indicates polymorphic position in the sequence context. All sequences are shown in 5' to 3' direction.

5

Table 13

| Polymorphic Site | Sequence | Note |
|------------------|---|----------------------------|
| 957 | SEQ ID NO:33: ATCTT <u>C</u> TATTG SEQ ID NO:34: ATCTTTTATTG | C variant T variant |
| 1049 | SEQ ID NO:35: ACAAT <u>A</u> GAAAG SEQ ID NO:36: ACAATGGAAG | A variant G variant |
| 1164 | SEQ ID NO:37: ATGGAG <u>A</u> AGGG SEQ ID NO:38: ATGGAAAAGGG | G variant A variant |
| 1526 | SEQ ID NO:39: TTAATG <u>G</u> TAAA SEQ ID NO:40: TTAATTGTAAA | G variant T variant |
| 1661 & 1662 | SEQ ID NO:41: GGATT <u>T</u> CATTAT SEQ ID NO:42: GGATTAAATTAT | TC variants AA variants |

Table 14 sets forth oligonucleotides representing the non-coding (anti-sense) strand complementary to the polymorphic region corresponding to the polymorphisms found in the study population. The underlined letter indicates polymorphic position in the sequence context. All sequences are shown in 5' to 3' direction.

10

Table 14

| Polymorphic Site | Sequence | Note |
|------------------|---|---|
| 957 | SEQ ID NO:43: CAATAG <u>A</u> AGAT SEQ ID NO:44: CAATAAAAGAT | Antisense G variant Antisense A variant |
| 1049 | SEQ ID NO:45: CTTTCT <u>T</u> ATTGT SEQ ID NO:46: CTTTCCATTGT | Antisense T variant Antisense C variant |
| 1164 | SEQ ID NO:47: CCCTT <u>C</u> TCCAT SEQ ID NO:48: CCCTTTTCCAT | Antisense C variant Antisense T variant |
| 1526 | SEQ ID NO:49: TTTAC <u>C</u> ATTAA SEQ ID NO:50: TTTACAATTAA | Antisense C variant Antisense A variant |
| 1661 & 1662 | SEQ ID NO:51: ATAATG <u>A</u> AATCC SEQ ID NO:52: ATAATTTAATCC | Antisense GA variants Antisense TT variant |

15

The sequences of Table 15 represent the 5'-sequence to the polymorphic sites on the coding (sense) strand (SEQ ID NO:s 53-57) and non-coding (anti-sense) strand (SEQ ID NO:s 58-67). All sequences are shown in 5' to 3' direction.

Table 15

| Polymorphic Site | Sequence | Note |
|------------------|--|--------------------------|
| 957 | SEQ ID NO:53: TACCTCCCATC SEQ ID NO:58: GATGGGAGGTA | Sense 5' Antisense 5' |
| 1049 | SEQ ID NO:54: AACCAAAAACA SEQ ID NO:59: TGTTTTTGGTT | Sense 5' Antisense 5' |
| 1164 | SEQ ID NO:55: CTGCAGTGATG SEQ ID NO:60: CATCACTGCAG | Sense 5' Antisense 5' |
| 1526 | SEQ ID NO:56: TAGGGGGTTTA SEQ ID NO:61: TAAACCCCCTA | Sense 5' Antisense 5' |
| 1661 & 1662 | SEQ ID NO:57: ATTTGAAAGGA SEQ ID NO:62: TCCTTTCAAAT | Sense 5' Antisense 5' |

- 5 The sequences of Table 16 represent the 3'-sequence to the polymorphic sites on the non-coding (anti-sense) strand (SEQ ID NO:s 63-67) and the coding (sense) strand (SEQ ID NO:s 68-72). All sequences are shown in 5' to 3' direction.

Table 16

| Polymorphic Site | Sequence | Note |
|------------------|--|--------------------------|
| 957 | SEQ ID NO:63: TGTGGATGCAA SEQ ID NO:68: TTGCATCCACA | Antisense 3' Sense 3' |
| 1049 | SEQ ID NO:64: CATGGCTGCTT SEQ ID NO:69: AAGCAGCCATG | Antisense 3' Sense 3' |
| 1164 | SEQ ID NO:65: AGGGATCTCCC SEQ ID NO:70: GGGAGATCCCT | Antisense 3' Sense 3' |
| 1526 | SEQ ID NO:66: TAAACACCTTT SEQ ID NO:71: AAAGGTGTTTA | Antisense 3' Sense 3' |
| 1661 & 1662 | SEQ ID NO:67: TGTTCTTTATA SEQ ID NO:72: TATAAAGAACA | Antisense 3' Sense 3' |

10

CLAIMS

1. An oligonucleotide primer pair suitable for amplifying a polymorphic region of a 5' flanking region of a *CYP3A4* gene, wherein the polymorphic region corresponds to position 816 of SEQ ID NO:1.

5

2. The primer pair of claim 1, having sequences selected from the group consisting of SEQ ID NO: 7 and SEQ ID NO:8 and SEQ ID NO:9 and SEQ ID NO:10.

3. A sequence determination oligonucleotide for detecting a polymorphic site in a 5' flanking region of a *CYP3A4* gene, said oligonucleotide being complementary to the polymorphic region corresponding to position 461 of SEQ ID NO:1.

4. The oligonucleotide of claim 3, comprising a sequence selected from the group consisting of SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID NO:17; and SEQ ID NO:18.

5. A kit comprising at least one oligonucleotide primer pair capable of amplifying the region corresponding to position 461 of SEQ ID NO:1.

6. The kit of claim 5, wherein the primer pair comprises sequences selected from the group consisting of SEQ ID NO: 7 and SEQ ID NO:8 and SEQ ID NO:9 and SEQ ID NO:10.

7. The kit of claim 5, further comprising a sequence determination oligonucleotide complementary to the polymorphic region corresponding to position 461 of SEQ ID NO:1.

8. The kit of claim 7, wherein the oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID NO:17; and SEQ ID NO:18.

9. An oligonucleotide primer pair suitable for amplifying a polymorphic region of a 5' flanking region of a *CYP2C9* gene, wherein the polymorphic region corresponds to position 957 of SEQ ID NO:6; position 1049 of SEQ ID NO:6; position 1164 of SEQ ID NO:6; position 1526 of SEQ ID NO:6; position 1661 of SEQ ID NO:6; and position 1662 of SEQ ID NO:6.

10. The primer pair of claim 9, having a sequence selected from the group consisting of SEQ ID NO: 19 and SEQ ID NO:20; SEQ ID NO:21 and SEQ ID NO:22; SEQ ID NO:23 and SEQ ID NO:24; SEQ ID NO:25 and SEQ ID NO:26; SEQ ID NO:27 and SEQ ID NO:28; SEQ ID NO:29 and SEQ ID NO:30; and SEQ ID NO:31 and SEQ ID NO:32.

11. A sequence determination oligonucleotide for detecting a polymorphic site in a 5' flanking region of a *CYP2C9* gene, said oligonucleotide comprising a sequence selected from the group consisting of an oligonucleotide complementary to the polymorphic region corresponding to position 957 of SEQ ID NO:6; an oligonucleotide complementary to the polymorphic region corresponding to position 1049 of SEQ ID NO:6; an oligonucleotide complementary to the polymorphic region corresponding to position 1164 of SEQ ID NO:6; an oligonucleotide complementary to the polymorphic region corresponding to position 1526 of SEQ ID NO:6; an oligonucleotide complementary to the polymorphic region corresponding to position 1661 of SEQ ID NO:6; and an oligonucleotide complementary to the polymorphic region corresponding to position 1662 of SEQ ID NO:6.

12. The oligonucleotide of claim 11, comprising a sequence selected from the group consisting of SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:39; SEQ ID NO:40; SEQ ID NO:41; SEQ ID NO:42; SEQ ID NO:43; SEQ ID NO:44; SEQ ID NO:45; SEQ ID NO:46; SEQ ID NO:47; SEQ ID NO:48; SEQ ID NO:49; SEQ ID NO:50; SEQ ID NO:51; SEQ ID NO:52; SEQ ID NO:53; SEQ ID NO:54; SEQ ID NO:55; SEQ ID NO:56; SEQ ID

NO:57; SEQ ID NO:58; SEQ ID NO:59; SEQ ID NO:60; SEQ ID NO:61; SEQ ID NO:62; SEQ ID NO:63; SEQ ID NO:64; SEQ ID NO:65; SEQ ID NO:66; SEQ ID NO:67 and SEQ ID NO:68.

- 5 13. A kit comprising at least one oligonucleotide primer pair, wherein the primer pair is capable of amplifying a polymorphic region selected from the group consisting of the polymorphic region corresponding to position 957 of SEQ ID NO:6; the polymorphic region corresponding to position 1049 of SEQ ID NO:6; the polymorphic region corresponding to position 1164 of SEQ ID NO:6; the polymorphic region
10 corresponding to position 1526 of SEQ ID NO:6; the polymorphic region corresponding to position 1661 of SEQ ID NO:6; and the polymorphic region corresponding to position 1662 of SEQ ID NO:6.

FIGURE 1

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1 CTGCAGTGAC CACTGCCCCA TCATTGCTGG CTGAGGTGGT TGGGGTCCAT CTGGCTATCT
61 GGGCAGCTGT TCTCTTCTCT CCTTTCTCTC CTGTTTCCAG ACATGCAGTA TTTCCAGAGA
121 GAAGGGGCCA CTCTTTGGCA AAGAACCTGT CTAAGTGGCT ATCTATGGCA GGACCTTTGA
181 AGGGTTCACA GGAAGCAGCA CAAATTGATA CTATTCCACC AAGCCATCAG CTCCATCTCA
241 TCCATGCCCT GTCTCTCCTT TAGGGGTCCC CTTGCCAACA GAATCACAGA GGACCAGCCT
301 GAAAGTGACAG AGACAGCAGC TGAGGCACAG CCAAGAGCTC TGGCTGTATT AATGACCTAA
361 GAAGTCACCA GAAAGTCAGA AGGATGCATA GCAGAGGCC AGCAATCTCA GCTAAGTCAA
421 CTCCACCAGC CTTTCTAGTT GCCCACTGTG TGTACAGCAC SCTGGTAGGG ACCAGAGCCA
481 TGACAGGGAA TAAGACTAGA CTATGCCCTT GAGGAGCTCA CCTCTGTTCA GGGAAACAGG
541 CGTGGAACA CAATGGTGGT AAAGAGGAAA GAGGACAATA GGATTGCATG AAGGGGATGG
601 AAAGTGCCCA GGGGAGGAAA TGGTTACATC TGTGTGAGGA GTTTGGTGAG GAAAGACTCT
661 AAGAGAAGGC TCTGTCTGTC TGGGTTTGGA AGGATGTGTA GGAGTCTTCT AGGGGGCACA
721 GGCACACTCC AGGCATAGGT AAAGATCTGT AGGTGTGGCT TGTGTTGGATG AATTTCAAGT
781 ATTTTGGAAT GAGGACAGCC ATAGAGACAA GGGCARGAGA GAGGCGATTT AATAGATTTT
841 ATGCCAATGG CTCCACTTGA GTTTCTGATA AGAACCCAGA ACCCTTGGAC TCCCCAGTAA
901 CATTGATTGA GTTGTTTATG ATACCTCATA GAATATGAAC TCAAAGGAGG TCAGTGAGTG
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1021 AGCAGAGGTG GCCCTGCTAC TGGCTGCAGC TCCAGCCCTG CCTCCTTCTC TAGCATATAA
1081 ACAATCCAAC AGCCTCACTG AATCACTGCT GTGCAGGGCA GGAAAGCTCC ATGCACATAG
1141 CCCAGCAAAG AGCAACACAG AGCTGAAAGG AAGACTCAGA GGAGAGAGAT AAGTAAGGAA
1201 AGTAGTGATG GCTCTCATCC CAGACTTGGC CATGGAAACC TGGCTTCTCC TGGCTGTCAG
1261 CCTGGTGCTC CTCTATCTGT GAGTAACTGT TCAGGCTCCT CTTCTCTGTT TCTTGACTT
1321 GGGGTCGTAA TCAGGCCTCT CTTTT

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FIGURE 2

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1  GATCTCAGAT ATCCCTTCTA TCTACACATT ATCTATAAATT CTTTCTTTCT GTAAACTGAA
61 AGGTCCTAGA AGGAGCCGCA GCTCAGCAGG AGAGAGGAGG AGCTGAGCTG GGACCCCTAC
121 CTCCTGAGGA ATGAAATGAT TATTATAAAG ACAGCAACCG AGCTTATTTT ACCCAAAATA
181 AGGTAGTATA TTTCTGTAG AGTTTAGAGT TTCATGAGTC AGGGACCAAG TTATTGCTTT
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361 TAATGTTTAT TTTGAAAATA ATTTACTAGA CTGAATTACG AAATCCTGAA TCATTGTACA
421 CTATCAGTAA ATATTGGTGG ACCCAACTGA ACTGAATGTT TTGCTTGAAA TGAAACCTTT
481 GAGATGCAGG GCTTATGGGT TCTAGTCCCA GCTCTAGCAC TAGCAGACAG CATGTTCTTG
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601 GTAAGCAGAG GTAATTGAGA GATTCAAAAG GGACATGAGG TGTAACAATT CTCTGTAAAT
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841 TATCTTTCTA GTTGTATTTA GATCCTCAAC TCAGTATGTC AGCTCCTGTT AAGGCTCTATA
901 CATTGTGGTG GTTCTGTGCT GTGGGTCCAT TTAGTGATTT CCCTACCTCC CATCTTTTAT
961 TGCATCCACA ACTGTGGTTC TGTCCATAAT TTCTTTTGCT TTCTGTGCAT TATTACATCA
1021 TATCTGAAAA TGAGAAACCA AAAACAATRG AAAGCAGCCA TGTCTGGAGG TGACTGGGGG
1081 GTCGAGAAGC CCTAGTTTCT CAAACCTTCA GCACCAAATT TTCCCTCAG TTACACTGAG
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 01/01580

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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| X | DATABASE EMBL 'Online! retrieved from EMBL, accession no. HS2C9X01 Database accession no. L16877 XP002229024 | 9-12 |
| Y | abstract | 9-13 |
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| Y | the whole document --- -/-- | 9-13 |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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- *O* document referring to an oral disclosure, use, exhibition or other means
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X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

30 January 2003

Date of mailing of the international search report

17/02/2003

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INTERNATIONAL SEARCH REPORT

Inter Application No

PCT/IB 01/01580

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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Information on patent family members

International Application No

PCT/IB 01/01580

| Patent document cited in search report | | Publication date | Patent family member(s) | Publication date |
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